THE UNIVERSITY OF ALBERTA

THE USE OF CERTAIN MICROBIAL INHIBITORS TO INCREASE THE KEEPING QUALITY OF COTTAGE CHEESE

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled The Use of Certain Microbial Inhibitors to Increase the Keeping Quality of Cottage Cheese submitted by Fen-lan Huang in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Cottage cheese is a nutritious food, but a highly perishable product because it provides an excellent medium for the growth of many microorganisms introduced during its manufacture.

It has a normal shelf life of 10 to 14 days at refrigeration temperatures in the market. However, occasionally the shelf life of the product is less than expected and this reduction is thought generally to be due to lapses in refrigeration during distribution or after purchase by the consumer. This study investigated the effects of exposure periods of as long as 8 hours at room temperature on the storage life of cottage cheese with and without microbial inhibitors.

The results showed significantly different effects for different inhibitors. Aureomycin and vitamin K_5 had little effect, sodium propionate had somewhat more effect and potassium sorbate had the greatest effect in extending the storage life of cottage cheese. Cottage cheese exposed to room temperature had a longer shelf life when the remainder of its storage was at $2^{o}C$ than when it was at 5^{o} or $8^{o}C$. Cottage cheese subjected to various room temperatures (i.e. $20-29^{o}C$) for periods of 4, 6 and 8 hours showed that the subsequent storage life varied inversely with the period of exposure.

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INTRODUCTION

1. Production of Cottage Cheese.

Cottage cheese is a popular food in the diet of many people. Production of cottage cheese has increased markedly over the past several years. In Canada, production of creamed and uncreamed cottage cheese in 1961 was 24 million pounds, in 1968 it was 34 million pounds (16,17). Important reasons for the increasing consumption of cottage cheese are its pleasant flavor, relatively low-caloric content, and relatively high content of protein, minerals and vitamins as shown in Table 1 (48).

The general procedure commonly used in the production of cottage cheese may be outlined as follows (21,27):

Pasteurized skim milk is inoculated with a suitable lactic starter and a coagulator such as rennet. If the milk is held at a temperature of 29.5° to $33.2^{\circ}C$ (85° to $92^{\circ}F$) lactic acid production will cause setting of the curd in four to five hours. This is the "short set" process which is widely used in commercial cheese production. In the "long set" process the milk is held at a temperature of 21° to $27^{\circ}C$ (70° to $80^{\circ}F$) and the curd forms in 12 to 16 hours. When a firm curd is formed, it is cut into small cubes. The time for cutting the curd is determined by the titratable acidity of the whey which conventionally is at or slightly above 0.50%. The curd is then slowly heated with

Table 1. Composition of creamed and uncreamed cottage cheese (48).

		Uncreamed cottage cheese	Cream	Creamed[a] cottage cheese
			per 100 g.	
Water	g.	79	80	78.3
Calories		87	136	104
Protein	g.	16.9	3.3	12.3
Fat	g.	0.4	12.0	4.3
Carbohydrate	g.	2.7	4.6	3.3
Calcium	mg.	90	107	96
Iron	mg.	0.4	0.04	0.3
Vitamin A	I.U.	9	492	173
Thiamine	mg.	0.03	0.03	0.03
Riboflavin	mg.	0.28	0.16	0.24
Niacin	mg.	0.09	0.04	0.07

a. Values calculated from those for cream and uncreamed curd; 1 lb. of cream added to 2 lb. of curd.

stirring, to about 49° to 54.5°C (120° to 130°F) and held at this temperature for about 15 minutes. This cooking is required to develop suitable curd firmness and is normally accomplished in about 2 hours. The whey is then drained from the curd, and the curd is immediately subjected to several washings with chilled water. Finally, the curd is drained. It is customary to add cream to the curd, whereupon it is packaged for storage and shipping.

For best quality cottage cheese, careful attention must be given to each step in the manufacturing process. Good quality skim milk should be used initially. The starter culture must be pure, and must be handled asceptically; temperature and pH must be controlled throughout processing; the water supply must be low in spoilage microorganisms and should be controlled to uniform pH. The finished product should be packaged in a room free from contamination by air-borne microorganisms (5,6,22,30,34).

Cottage cheese sold to the consumer should have a slightly acid, salty flavor and should have the delicate aroma of a good lactic acid starter (2). If cottage cheese is manufactured from high quality products and is carefully handled, it can be expected to have minimum flavor deterioration during storage for from one to two weeks at 5.5°C (42°F). Harmon et al (24) have shown that desirable shelf life is attained when cottage cheese is stored at 5.5°C. They found that at a storage temperature of 10°C, shelf life ranged from 5 to 16 days and averaged 10.9 days.

While at 5.5°C shelf life ranged from 5 to 23 days and averaged 16.5 days. Under less satisfactory storage conditions the product may undergo extensive loss of flavor in three to five days. On the other hand, if it is held at 2.2° to 4.4°C (36° to 40°F) its storage life may extend to about three weeks.

2. Causes of Poor Storage Life.

Spoilage of cottage cheese is usually brought on by microbial invasions which produce chemical changes causing the development of fruity, putrid flavors and slimy surface growth (2,19,46).

It is compulsory to pasteurize the skim milk used in making cottage cheese and the high cooking temperatures for curd at low pH of 4.55 to 4.68 usually make it safe to assume that at the time of draining the whey the curd is relatively free of thermoduric organisms.

Contamination after this stage may originate from water, air, cream dressing, unclean utensils and general unsanitary practices. Harper *et al* (26) have shown that all starter organisms were destroyed rapidly at 49°C (120°F) and, even though there were differences in the rate of destruction, 99% kill of all starter organisms occurred in 15 minutes (Figure 1). Consequently, poor storage life in the finished product would primarily result from the survival of thermoduric organisms in the milk supply or post-cooking contamination.

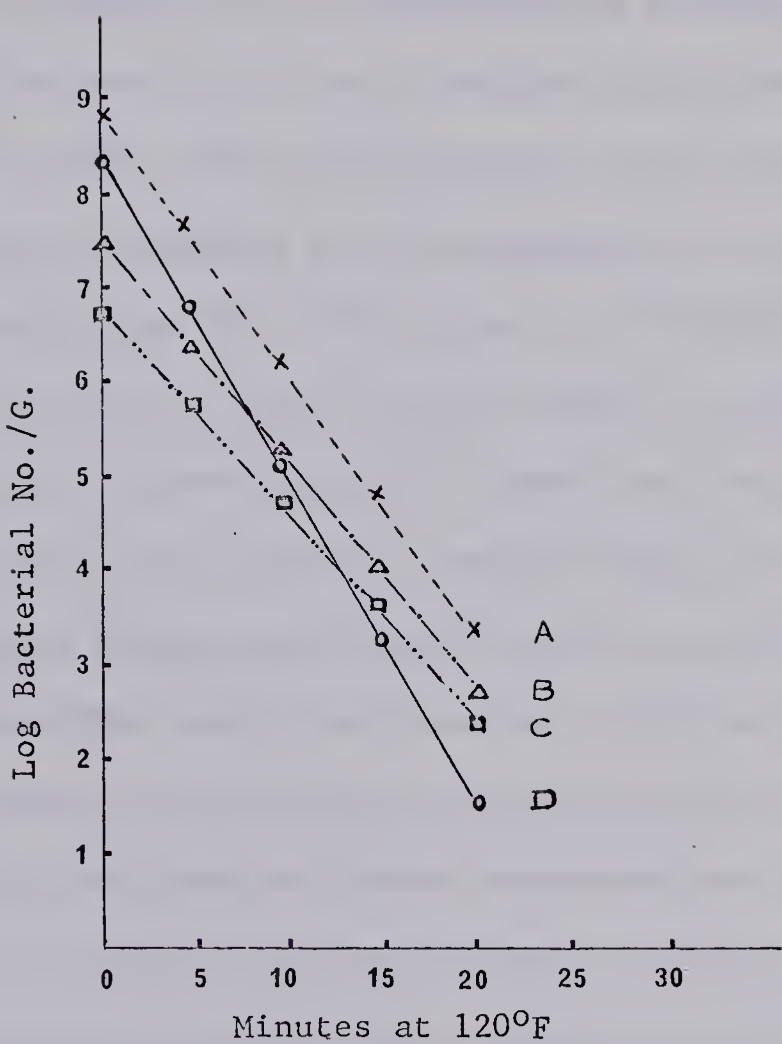

Figure 1. Effect of cooking cottage curd at 120°F on the destruction of bacteria (from Harper and Randolph, 26)

A, B, C, D : Four kinds of starter cultures used for commercial cottage cheese making.

Reference has been made to the observations of Harmon _et al_ (24) respecting the effect of temperature on storage life. Elliker (19) also reported that storage defects developed slowly at 4.4°C (40°F) or lower, but that rapid spoilage occurred at 10° to 15.5°C (50° to 60°F). Davis and Babel (13) found that the rate of slime formation was related to the temperature, and the slime appeared more rapidly at 10°C (50°F) than at 4.4°C (40°F).

Poor storage life is also directly related to the pH of the finished product. Harmon _et al_ (24) found that cottage cheese samples having a pH above 5.0 had an average shelf life of 13.5 days, while samples having a pH below 5.0 had an average shelf life of 16.0 days. The types of spoilage and off-flavor that accompany deterioration in storage are also related to changes in pH. For example the unclean flavors associated with high coliform counts occurred at high pH, but not at low pH for most samples. Yeasty flavor was pronounced at pH 4.42 to 4.89, while putrid and bitter flavors associated with proteolytic organisms occurred at all pHs, but were more common above 5.0 (35).

As an additional cause of spoilage, the fat of cottage cheese can undergo oxidative and hydrolytic changes resulting in flavor defects. Angevin (3) has reported that the butter fat content of cottage cheese may be varied according to the method of manufacture, size of curd, firmness of curd, etc., and usually ranges from 11 to 17%. Rhodes _et al_ (39) reported

four-fold and higher increases in free fatty acids during 7 days storage. Stull et al (45,46) have shown that auto-oxidation can be retarded by the antioxidant nordihydroguaiaretic acid (NDGA) and that its inclusion in cottage cheese dressing will extend the storage life.

3. Spoilage Organisms Causing Reduced Storage Life.

The primary cause of poor storage life of cottage cheese must be attributed to post-pasteurization and post-cooking contamination (3,13,39). The microbial populations which may be responsible for poor storage life can be classified into three major groups (a) coliforms, (b) yeasts and molds, (c) psychrophiles.

Coliforms: The presence of coliform organisms in large numbers in cottage cheese is important, not primarily because they are harmful in themselves but because they indicate negligent practices in the plant. When present in large numbers these organisms impart a rotten flavor (indol) to cottage cheese. Although some individuals associate these organisms with faecal contamination, in the milk industry coliforms may arise from other sources. These organisms are capable of growing in many places and under a wide range of conditions, generally not related to the colon of an animal. For example the coliform *Escherichia coli* and *Aerobacter aerogenes* may be found growing on unsterilized equipment such as pails, vats, pipes, milking

machines and other plant equipment. Coliform organisms can usually be controlled by the following practices:

(a) Cook the curd at 49^{o} to $54.5^{o}C$ ($120^{o}$to $130^{o}F$) for 20 to 30 minutes.

(b) Sterilize all equipment and carry out all operations under strict sanitary conditions.

(c) Make sure that the cream added is coliform-free.

(d) Store the creamed cottage cheese at 2.2^{o} to $4.4^{o}C$ (36^{o} to $40^{o}F$).(47).

Yeasts and molds: Bonner *et al* (8) isolated two species of yeasts and three species of molds from spoiled cottage cheese. Harmon (25) has shown that the molds most commonly found in cottage cheese are (a) *Geotrichum candidum* which produces an off-white or tan to yellow color, (b) a filamentous gray species of *Mucor* and (c) the blue-green *Penicillium*. Molds cause discoloration, staleness or moldy flavors. Through their utilization of lactic acid the pH of the cheese may be raised to levels that enhance the development of spoilage bacteria (19). Yeasts most frequently found are species of *Rhodotorula* which produce vivid pink spots and pink slime and *Torulopsis* spp. which produce a yellow slime. Yeasts ferment lactose and produce yeasty, fruity or acid flavor and sometimes excessive gas (19,25).

Flavor defects in cottage cheese produced by spoilage organisms are often described as bitter, rancid, musty, stale,

unclean, fruity, cheesy, and rotten. Sometimes such flavor defects may be accompanied by physical changes such as gelatinous or "tapioca" curd formation. Invariably, excessive growth of bacteria precede the organoleptic defects found in cottage cheese, and the flavor change when it comes may occur suddenly within one day (19).

Psychrophiles: This group includes the organisms which are perhaps the most troublesome. According to Harmon (25) they are largely restricted to the genera *Pseudomonas*, *Alcaligenes*, *Flavobacterium* and *Achromobacter*. They produce pigmented slime or gelatinous films along with objectionable flavors and odors.

Elliker (19) has shown that the most offensive species which have received extensive study are the following:

(a) *Ps. viscosa* which produces a yellowish or brownish colored slimy film accompanied by flat bitter or putrid flavor and rotten flavor,

(b) *Ps. fragi* which imparts a fruity odor, a flat rancid or bitter flavor and a white gelatinous film on the surface of curd particles,

(c) *Alcaligenes metalcaligenes* which forms a white gelatinous film, but produces little changes in flavor or odor.

Sandine *et al* (40) have reported that the first spoilage defect associated with these low temperature organisms is the loss of flavor and aroma. Parker *et al* (36,37) reported on

the ability of psychrophilic bacteria to destroy biacetyl, an important flavor compound in cottage cheese. It was Seitz _et al_ (41) who showed that these bacteria contain a specific enzyme which rapidly converts biacetyl to flavorless and odorless compounds. It is clearly seen how psychrophiles are truly a hindrance to extended storage life. They receive no competition from starter bacteria, since the latter are lost in the whey during draining and washing, thus psychrophilic growth is uninhibited, and accordingly, they cause spoilage and consequent reduction of storage life of cottage cheese.

4. _Origin of Spoilage Bacteria_.

The most common sources of spoilage bacteria are soil and water (18). Harmon (25) suggested water as the primary source of spoilage bacteria. He showed that cheese washed with contaminated water will become thoroughly and uniformly contaminated. These organisms are non-fastidious in their nutritive requirements and may survive in large numbers in storage water vats. Organic sediment found in reservoirs forms a good substrate for harbouring them. Water supplies examined in cottage cheese plants have revealed thousands of psychrophiles per ml. Sections of hose used to run cold water into vats or churns are submerged in the whey, thus allowing spoilage bacteria easy access to plant water systems. Contamination from air, dust and condensation is a significant harzard to keeping quality. Martin _et al_ (31) have suggested that the finished product should be free

from harmful microorganisms if proper processing techniques and controls are followed, however, contamination from workers hands, clothes, and impure wash water or added cream all make cottage cheese a perishable product during prolonged storage.

5. Controlling Spoilage Bacteria to Enhance Storage Life.

One of the important ways to enhance storage life of cottage cheese is to prevent the rapid growth of microorganisms by keeping the product at low storage temperatures. Microbial growth is well known to be retarded at temperatures below 4.4°C (40°F). Collins (11) demonstrated that low temperature (3.5°C or 38.3°F) was very effective as a means of retarding the development of fruityness which was caused by the growth of *Pseudomonas fragi*, the defect that most commonly limited the storage life of commercial cottage cheese.

Additions of several chemicals and antibiotics have also been studied as a means of extending storage life. Wyss *et al* (50) found that the inhibitory activity of a fatty acid increased with decreasing pH and that unsaturated fatty acid are more inhibitory than the saturated acids. Keeney (28) reported fungicidal properties for the 5, 6, 8, 10 and 11 carbon fatty acids, whereas, the shorter chain acids exhibited only fungistatic properties. Bell *et al* (7) reported that sorbic acid was an effective deterrent of growth for many species of bacteria, yeasts and molds in nutrient media ranging in pH from 7.0 to

4.5. At pH 4.8, 50% of the acid is undissociated; thus, at this pH, only one half of the total sorbic acid would be effective.

The ability of potassium sorbate to inhibit surface spoilage organisms and the capability of sorbic acid to extend the normal storage of cottage cheese have been demonstrated by many workers (9,10,38). However, they found that a flavor hazard placed a limitation in the use of sorbic acid. An off-flavor was frequently present in samples of cheese containing 0.100% sorbate but not in cheese containing 0.075%.

Giminder (23) showed that sorbic acid, sodium sorbate and potassium sorbate at 0.05% to 0.075% extended the shelf life of non-acid-washed cottage cheese by retarding yeast and mold growth. The shelf life of the cottage cheese treated with 0.075% sorbic acid or potassium sorbate averaged 30 days while the shelf life of the control sample averaged 13 days. Collins et al (12) showed that potassium sorbate (0.05% or more) added to cottage cheese retarded the growth of bacteria that produced slime and fruity and putrid odors at refrigeration temperatures, bacteria that produced sourness (probably lactic streptococci), and molds. An addition of 0.05% or more of potassium sorbate prolonged the shelf life of cottage cheese beyond 45 days.

Other chemicals and antibiotics such as vitamin K_5, Aureomycin, Pimaricin have been tested for their effects to enhance the shelf life of cottage cheese. Yang (51) showed

that vitamin K_5 at 20 ppm effectively controlled mold growth in cottage cheese for 28 days at 3.3°C (38°F) and for 7 days at 22.2°C (72°F). Olson et al (33) and Kristoffersen et al (29) have shown Aureomycin was generally effective in controlling bacterial spoilage in cottage cheese.

Shahani et al (32,42) have shown that at 4.4°, 10° and 15.5°C (40°, 50° and 60°F) untreated cheese spoiled and became unmarketable within an average of 16.8, 10 and 7.3 days respectively while samples treated with 50 ppm of Pimaricin became unmarketable in 26.6, 22 and 15 days respectively. The antibiotic treatment improved the keeping quality by approximately 10, 12 and 8 days respectively. It showed that the antibiotic treatment improved the keeping quality of cottage cheese considerably at lower temperatures of 4.4° and 10°C (40° and 50°F), and little or not at all at the higher temperature 15.5°C (60°F).

Harmon et al (24) have shown that the shelf life of cottage cheese can be prolonged in low temperature cabinet storage and the storage life was longer at a uniform temperature of 5.5°C (42°F) than at the fluctuating temperature to which the product is exposed in retail markets and home storage.

During the distribution, and after it has been purchased by the consumer, the product may be exposed to undesirably high temperatures either because of equipment failures or a lack of care by personnel handling the product. Such heat shock has

been reported to reduce the storage life of the product and hasten the onset of spoilage. Although several reports have been found regarding the use of microbial inhibitors to extend the storage life of cottage cheese held continously at various temperatures, a search of the literature has revealed no reports on the use of inhibitors to overcome the effects of periods of "heat shock" during the storage of cottage cheese. In order to protect against losses under conditions of heat shock and to extend the normal storage life, processors have suggested that microbial inhibitors might be useful additives for cottage cheese. Although such additives are not permitted under present regulations it has been considered that their use should be investigated. Accordingly, this investigation was planned and undertaken to learn the effects of certain microbial inhibitors to extend the storage life of cottage cheese held at different storage temperatures and, particularly, to overcome the harmful effects of a heat shock of as long as eight hours at ambient temperature.

MATERIALS AND METHODS

1. Product Preparation.

Portions of curd and cream were separately taken from the regular production of cottage cheese in a local dairy. The curd was made using the "short set" method from good quality skim milk fortified to 8.5 – 10.5% solids-non-fat (SNF) with low heat non-fat dry milk, and pasteurized at 71.1° to $72.2^{\circ}C$ (160° to $162^{\circ}F$) for 16 to 18 seconds. Five per cent of active starter was added to the pasteurized skim milk. Forty-five minutes later, 28 to 29 ml of coagulator was added per 1000 lb. and mixed thoroughly into the skim milk. The curd was cut (3/8 inch) at a whey titratable acidity of 0.55% (pH 4.7), and heated to $49^{\circ}C$ ($120^{\circ}F$) within 1.5 to 2 hours and held at this temperature for approximately 15 minutes until a desirable consistency was attained. After draining, the curd was washed three times using acidified water at pH 5.0 to 5.2. The last wash consisted of water at $4.4^{\circ}C$ ($40^{\circ}F$) containing not less than 4 ppm of available iodine (IOBAC* germicide solution) which left an undetectable amount of iodine after draining. The finished curd contained not less than 20% total solids and was ready for creaming or storage.

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* IOBAC: Iodine germicide. Manufactured by Lazarus Laboratories, Chemical Products, Ltd., Montreal, Canada.

The creaming mixture was prepared by the addition of 0.33% STA-RITE** stabilizer to cream of 10.0 to 10.5% fat, pasteurizing at 75.5 to 76.4°C (168°to 170°F) for 16 seconds, homogenizing under pressures of 7,000 to 12,000 pounds per square inch and cooling to 4.4°C (40°F).

2. Experimental Methods.

In the laboratory, prior to the creaming operation, appropriate inhibitors (potassium sorbate, sodium propionate, Aureomycin and vitamin K_5), each at certain levels were dissolved in 15 to 20 ml of sterilized distilled water. One per cent of salt and the inhibitor solutions were mixed into the cream which was then added to the curd in the proportion of one part of cream to two parts of curd. The product was well mixed and packed in portions of 10 to 12 ozs in waxed paper containers. At least 10 cartons of cream cottage cheese were prepared for each treatment. Cartons were selected from each treatment at random for tests as indicated below.

Eight trials were conducted. In Trials 1 to 5, the shelf life of the products containing inhibitors was compared with non-treated controls. In Trial 6, the variablity

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** STA-RITE: Manufactured by Germantown Manufacturing Co., Philadelphia 31, Pa., U.S.A.

was determined in microbial counts between randomly chosen cartons from single lots of cottage cheese under the conditions used in this study. In Trials of 7 and 8, changes were studied in the water soluble nitrogen content of cottage cheese with and without inhibitors. Samples were stored at $2 \pm 1^{\circ}$ and $5 \pm 0.5^{\circ}C$ in the first trial, at $2 \pm 1^{\circ}$, $5 \pm 0.5^{\circ}$ and $8 \pm 2^{\circ}C$ in second, third and fourth trials, at $5 \pm 0.5^{\circ}C$ in the remaining trials except for a period of time when they were exposed to room temperature to provide a "heat shock". The heat shock was provided on the second day in seven of the trials. In the fifth trial, different samples were exposed to heat shocks on the second, fourth and sixth days.

Detailed descriptions of test conditions for each trial are as follows:

<u>Trial 1</u>.

Potassium sorbate (0.075%) and sodium propionate (0.080%) were used as inhibitors. Portions of each lot were stored at 2° and $5^{\circ}C$ except for the period of 4 hours on the second day after creaming when all samples were placed individually at room temperature ($23.5^{\circ}$to $24.5^{\circ}C$). This caused the temperature of the cottage cheese to reach 20 $^{\circ}C$ after 4 hours exposure.

Trial 2.

Vitamin K_5 (15 ppm) and Aureomycin (6 ppm) were used as inhibitors. Portions of each lot were stored at 2^o, 5^o and 8^oC except for the period of 8 hours on the second day after creaming when they were exposed to room temperature (26.8^o to 28.5^oC). The temperature of cottage cheese reached the room temperature after 6 hours exposure.

Trial 3.

Four inhibitors, each at two levels: potassium sorbate (0.075% and 0.100%), sodium propionate (0.075% and 0.100%), Aureomycin (6 ppm and 9 ppm)and vitamin K_5 (15 ppm and 20 ppm) were used as inhibitors.

Two lots, C_1 and C_2, were maintained as controls. Lot C_1 exposed to room temperature (26.5^o to 29.5^oC) with the test samples, served as an intra-trial control while lot C_2, kept continuously at 5^oC, served as an inter-trial control and as a base for measurement of the heat shock effects in this trial.

Portions of each test lot and the control were stored at 2^o, 5^o and 8^oC interrupted by periods of 4, 6 or 8 hours of exposure to room temperature. Portions otherwise stored at 2^o and 8^oC received each exposure and portions otherwise stored at 5^oC received only 8 hours of exposure.

Trial 4.

One lot of potassium sorbate (0.05%) and two lots of control samples were prepared. One control lot (C_2) was held continuously at $5^{o}C$ while the other control and the test samples were held at 2^{o}, 5^{o} and $8^{o}C$ except for periods of exposure to room temperature (25^{o} to $28.5^{o}C$) for 4, 6 and 8 hours on the second day after creaming. In these exposures the temperature of the cottage cheese reached $28.5^{o}C$ after 6 hours.

Trial 5.

Three lots of cottage cheese were prepared: one containing potassium sorbate (0.05%), one containing sodium propionate (0.05%) and one containing no inhibitor. All lots were stored at $5^{o}C$ except for an 8-hour exposure to room temperature on the second, the fourth or the sixth day after creaming. Temperatures of 23.5^{o}, 24^{o} and $25.9^{o}C$ respectively were reached in samples exposed.

Trial 6.

Two lots of creamed cottage cheese were prepared: one was a control lot with no inhibitor and the other lot was treated with potassium sorbate (0.05%). Portions of dry curd and cream made two additional lots for tests. For each lot, 25 cartons were prepared and all cartons were held at $5^{o}C$ except for an 8-hour period of exposure at room temperature (23.7^{o} to $25.3^{o}C$)

on the second day. During this exposure the temperature of the samples rose from 4.5^{o} to $24.2^{o}C$. Microbial tests were carried out initially and after one and two weeks when the customary four counts were conducted on three randomly chosen cartons from each lot.

Trials 7 and 8.

Each trial was conducted with two lots of cottage cheese, one an untreated control, the other, a lot to which 0.05% potassium sorbate had been added. Both lots were stored at $5^{o}C$ except for an 8-hour period of exposure on the second day after creaming during which the temperature of the cottage cheese reached $25.5^{o}C$ and $26.8^{o}C$ in the seventh and eighth trials respectively.

The tests performed in the various trials were as follows:

Trials 1, 2 and 3	:	Microbial test, titratable acidity and pH test.
Trial 4	:	Microbial test.
Trial 5	:	Microbial test and pH test.
Trial 6	:	Microbial test.
Trials 7 and 8	:	Water soluble nitrogen and total nitrogen determination.

A detailed description of each test is given below.

A. Microbial Counts (1)

Samples were mixed in the original container with a sterile Waring Blender. An equal weight of sterile stock phosphate buffer (pH 7.2) was added the mixture was agitated for 1 to 1.5 minute intervals with 30 second interspaced stops to allow curd particles to contact the knife for more effective blending. This procedure satisfactorily disintegrated the curd. The procedures of microbial tests were immediately carried out.

(a) Standard Plate Count (SPC)

The standard plate count was determined using standard plate count agar (Difco) and incubation at 32°C for 48 hours.

(b) Coliform Counts

Samples were plated in Violet red bile agar (Difco) and incubated at 37°C for 24 hours.

(c) Yeast and Mold Counts

Potato dextrose agar (Difco), acidified to pH 3.5 with tartaric acid (10% solution), was used as the medium. Incubation was at room temperature for 5 days.

(d) Psychrophile Counts

Psychrophile counts were made in standard plate count agar (Difco) with incubation at 5°C for 7 days.

B. Titratable Acidity and pH Test (1)

The titratable acidity was determined by titrating a 9 gram sample of cheese diluted with 9 ml. distilled water, and three drops of phenolphthalein (1%) to a permanent pink end point with 0.1N sodium hydroxide. Duplicate determinations were made. The pH was determined with a Beckman glass electrode pH meter Model H2. Readings were taken in duplicate after immersing the electrodes directly into the sample of triturated cheese.

C. Soluble Nitrogen (SN) and Total Nitrogen (TN) Determination (44).

A 150 to 200-gram sample of cottage cheese was mixed in a Waring Blender and analyzed for total nitrogen by the Kjeldahl method (4). The soluble nitrogen was extracted by a modification of the procedure of Vakaleris and Price (49). To five grams of the blended cheese, 10 ml of water and two drops of 1% pheno-phthalein indicator were added. Sufficient 0.2N NaOH was added to maintain a faint pink color and the solution was adjusted with a pH meter to pH 4.4 by adding 0.2N HCl. The mixture was then centrifuged for 5 minutes at 1,400 g and the supernatant filtered through Whatman No. 1 paper. The precipitate was rinsed with dilute HCl (pH4.5), centrifuged, and filtered. The combined filtrates (containing the soluble nitrogen compounds) were diluted to 100 ml, and 15 ml aliquots were analyzed for nitrogen by the Kjeldahl procedure.

The soluble nitrogen was expressed as per cent of total nitrogen as follows:

$$\text{SN (\%)} = \frac{\text{100 x mg of SN extracted from 1 g of cheese}}{\text{mg of TN in 1 g of the cheese}}$$

D. Indication of the End of Shelf Life.

The samples were observed for evidence of microbial growth and flavor changes. Five cartons from each treatment chosen at random were allocated for these examinations. Usually evidence of growth was taken to indicate the end of storage life but in cases when this was delayed, a loss of acceptable flavor or the development of off-flavor determined organoleptically became the indicator.

RESULTS

1. Inhibitor Effects.

In Trial 3 (Figure 2), cottage cheese treated with potassium sorbate kept for 48 days at $2^{o}C$ and $5^{o}C$ and for 30 days at $8^{o}C$, while control samples kept for only 3 weeks at $2^{o}C$, 2 weeks at $5^{o}C$, and 1.5 weeks at $8^{o}C$. Since all test samples had at least the shelf life of the control sample C_2 which was stored continuously at $5^{o}C$ (16.8 days) it was apparent that all the inhibitor treatments successfully overcame the effect of an exposure period as long as 8 hours. In addition, the sodium propionate, and particularly the potassium sorbate treaments, more than compensated for this loss in storage life. The superiority of the performance of potassium sorbate and sodium propionate over that of Aureomycin and vitamin K_5 are also indicated in Trials 1, 2 and 5 (Figures 3, 4 and 5).

For each inhibitor tested, differencesin the effects on keeping quality resulting from different concentrations used were not significant (Table 2).

2. Storage Temperature Effects.

In Trials 2 and 3 (Figure 2 and 4), control samples stored at 2^{o}, $5^{o}$and $8^{o}C$ kept for 20, 15 and 9 days respectively when they were exposed to room temperature for 8 hours on the second day. Antibiotic-treated samples under the same conditions kept

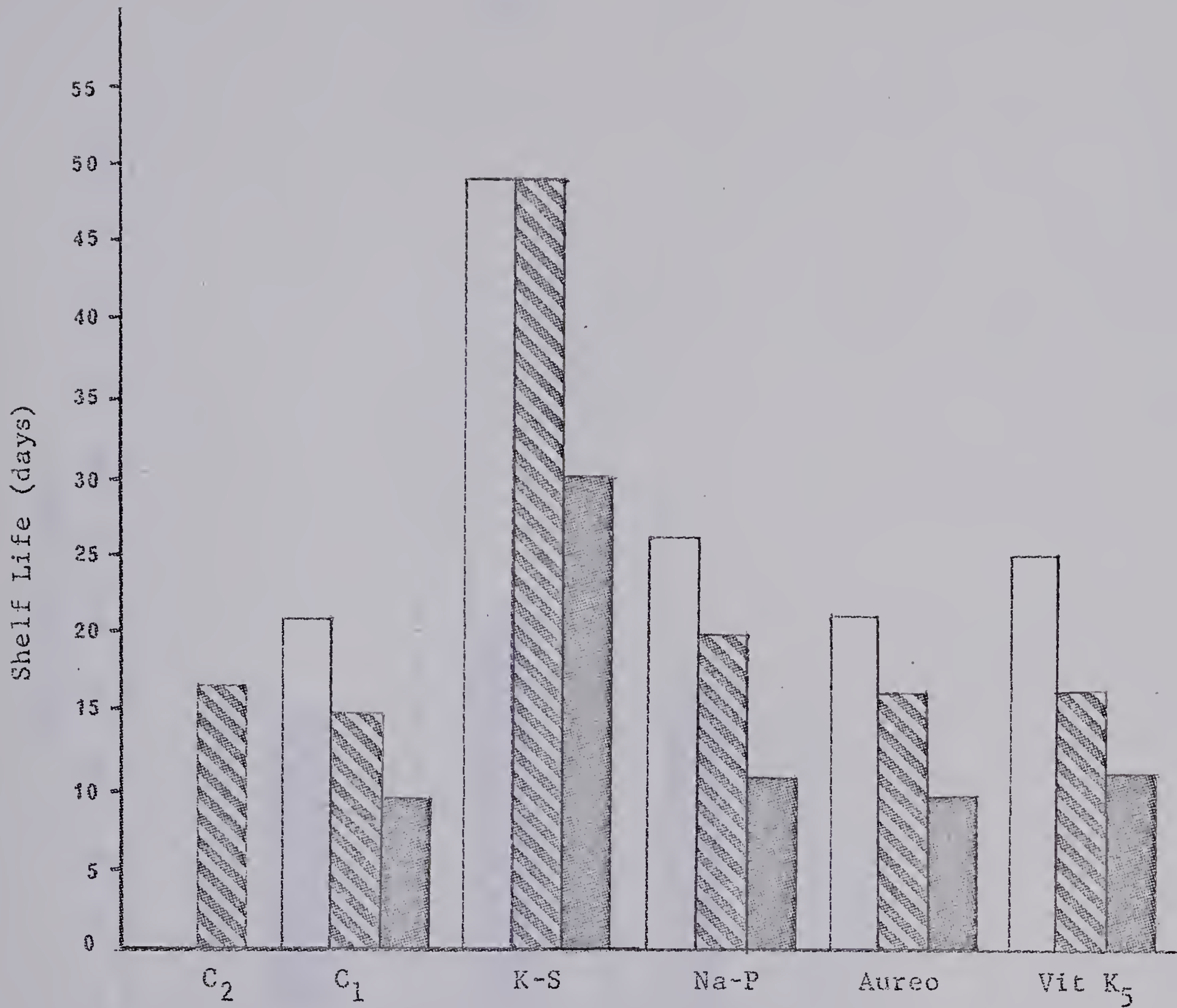


Figure 2. Shelf life of cottage cheese exposed to room temperature for 8 hours on the second day after manufacture and otherwise stored at 2°, 5° and 8°C (Trial 3).

C_2 : Control (not exposed)

C_1 : Control (exposed)

K-S : Potassium sorbate 0.075%

Na-P : Sodium propionate 0.075%

Aureo : Aureomycin 6 ppm

Vit K_5 : Vitamin K_5 15 ppm

 : 2°C

 : 5°C

 : 8°C

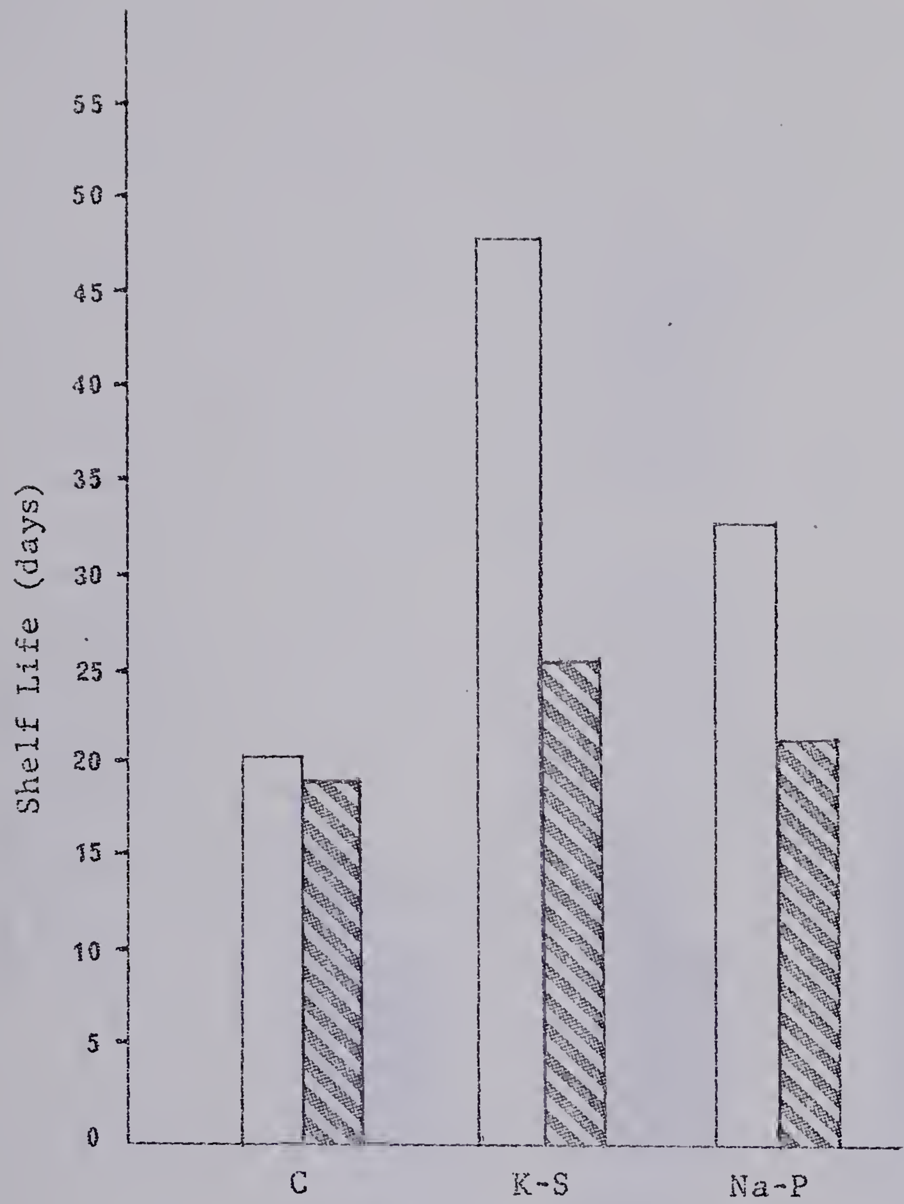


Figure 3. Shelf life of cottage cheese exposed to room temperature for 4 hours on the second day after manufacture and otherwise stored at 2° and 5°C (Trial 1).

C : Control

K-S : Potassium sorbate 0.075%

Na-P : Sodium propionate 0.080%

☐ : 2°C

▨ : 5°C

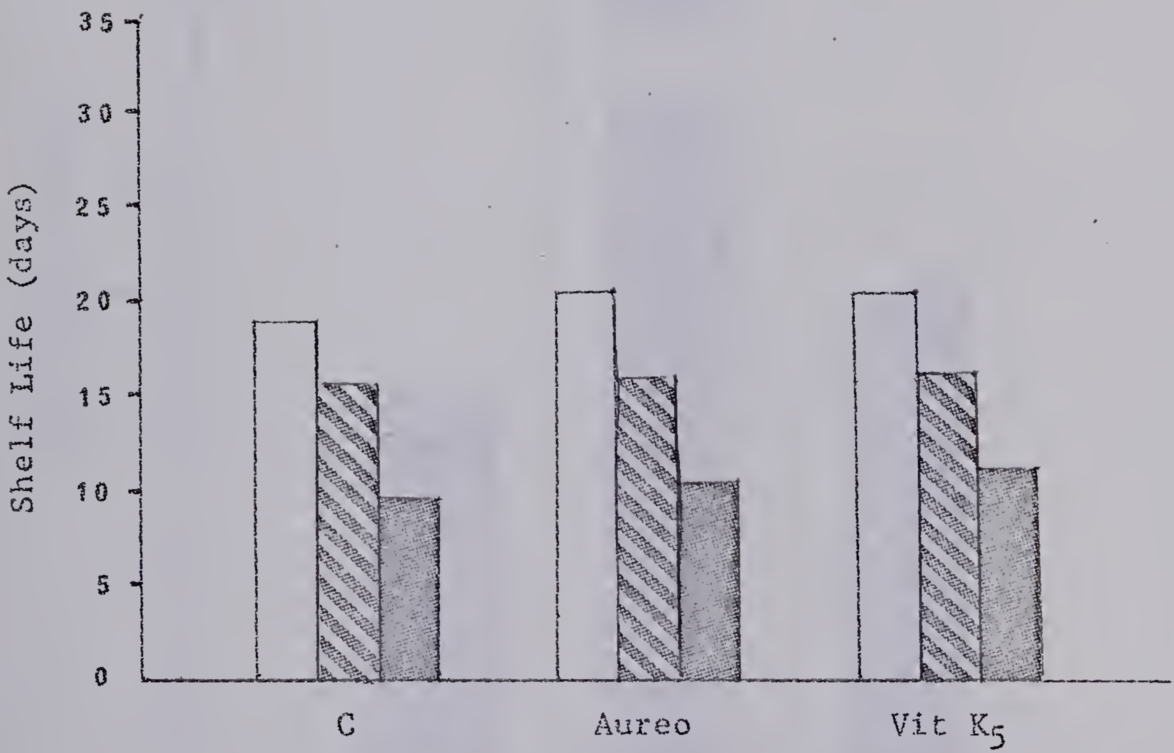


Figure 4. Shelf life of cottage cheese exposed to room temperature for 8 hours on the second day after manufacture and otherwise stored at 2^o, 5^o and 8^oC (Trial 2).

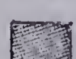
C : Control

Aureo : Aureomycin 6 ppm

Vit K_5 : Vitamin K_5 15 ppm

 : 2^oC

 : 5^oC

 : 8^oC

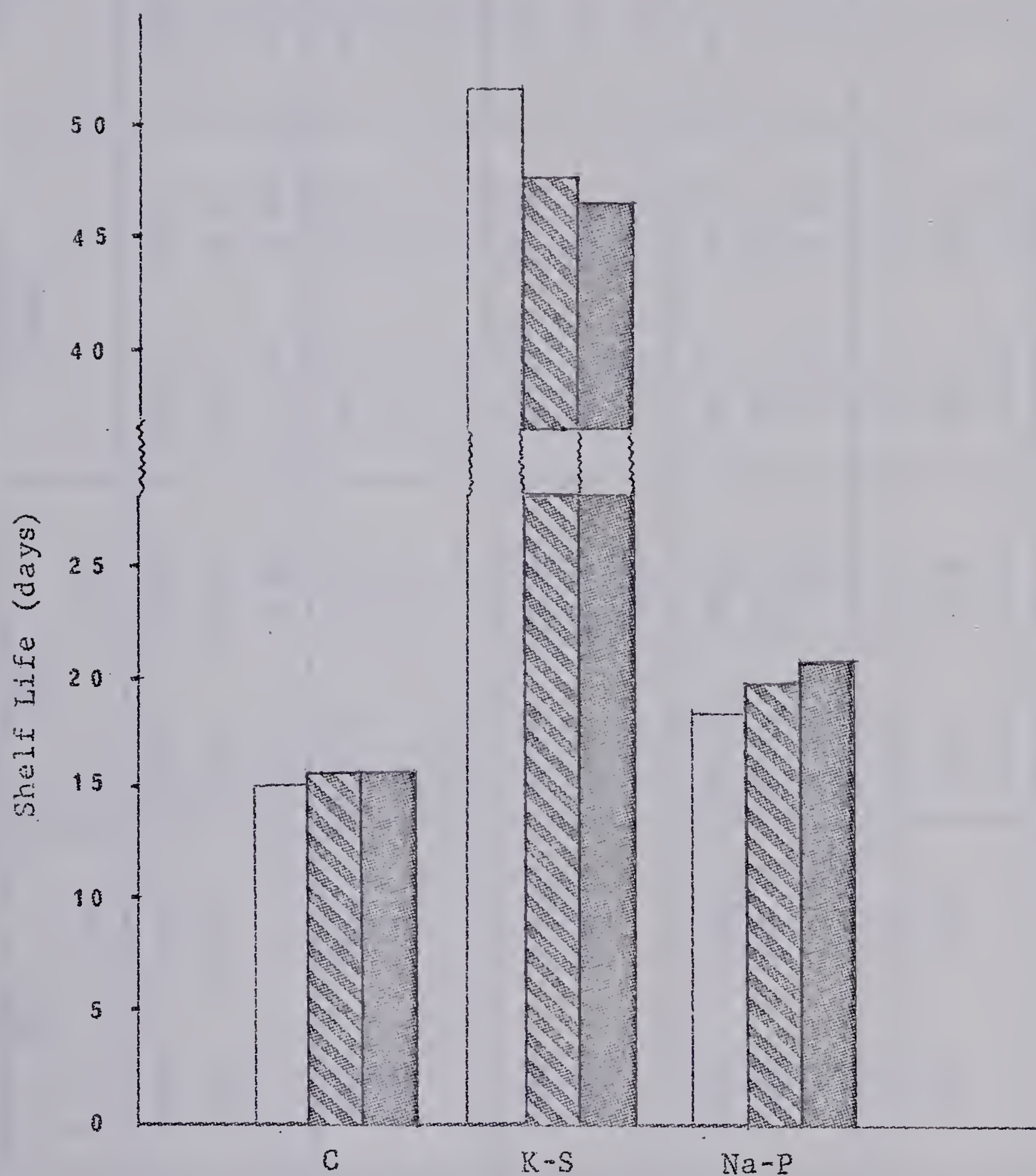


Figure 5. Shelf life of cottage cheese exposed to room temperature for 8 hours on different days after manufacture and otherwise stored at 5°C (Trial 5).

C : Control

K-S : Potassium sorbate 0.050%

Na-P : Sodium propionate 0.050%

 : Second day

 : Fourth day

 : Sixth day

Table 2. Periods of time in days to show evidence of microbial growth (Trial 3).
S : Standard deviation from the mean.

Storage Temp. (C.)	Heat Shock (hr.)	Treat-ments	Control C_1	Control C_2	Potassium-sorbate (%) 0.075	Potassium-sorbate (%) 0.100	Sodium-propionate (%) 0.075	Sodium-propionate (%) 0.100	Aureomycin (ppm) 6	Aureomycin (ppm) 9	Vitamin K_5 (ppm) 15	Vitamin K_5 (ppm) 20
2°	4	range	22-24		> 48	> 48	32-33	33-35	33-35	33-35	28-30	28-30
		mean	22.8		> 48	>48	32.6	34.2	34.0	33.8	29.2	28.8
		S	o.84				0.55	0.84	1.00	0.84	0.84	0.84
	6	range	21-24		>48	>48	27-30	27-30	24-27	27-30	24-26	27-28
		mean	22.2		>48	>48	28.8	28.6	25.2	28.2	25.0	27.6
		S	1.30				1.30	1.34	1.30	1.30	1.00	0.55
	8	range	21-24		>48	>48	26-28	24-25	23-24	23-24	25-27	24-27
		mean	22.4		>48	>48	26.8	24.6	23.4	23.6	26.2	25.6
		S	1.34				0.84	0.55	0.55	0.55	0.84	0.55
5°	8	range	14-15	16-17	>48	>48	18-22	18-23	16-17	16-17	16-18	16-17
		mean	14.6	16.8	>48	>48	20.0	20.2	16.4	16.8	17.2	14.6
		S	o.55	0.45			1.59	3.80	0.55	0.45	0.55	0.55
8°	4	range	12-13		>30	>30	16-17	15-17	15-17	12-13	14-16	14-15
		mean	12.4		>30	>30	16.4	15.8	15.8	12.6	15.4	14.6
		S	0.55				0.55	0.84	0.84	0.55	0.89	0.55
	6	range	11-12		>30	>30	13-14	13-14	13-14	11-12	13-15	14-15
		mean	11.2		>30	>30	13.6	13.4	13.4	11.8	13.4	13.8
		S	0.45				0.55	0.55	0.55	0.45	0.55	0.84
	8	reange	9-10		>30	>30	12-13	13-14	10-11	9-10	12-13	12-13
		mean	9.4		>30	>30	12.4	13.6	10.4	9.8	12.6	12.8
		S	0.55				0.55	0.55	0.55	0.45	0.55	0.45

for 24, 17, 10 days (Aureomycin) and 26, 17, 13 days (vitamin K_5).

The differences in shelf life resulting from differences in storage temperatures (from 2^o to 5^o to 8^oC) varied with the treatment but in all cases lower storage temperature markedly extended the shelf life of cottage cheese. The same effect of temperature on storage life was observed in Trials 1 and 4.

3. Exposure Effects.

As indicated in Trial 3 (Table 2), the shelf life of control sample C_1 (held at 5^oC except for an 8-hour exposure to room temperature) was approximately 2 days less than that of control sample C_2 (held continuously at 5^oC). This difference, due to an 8-hour exposure, was statistically highly significant (Appendix A).

Control samples C_1 exposed to room temperature for 4, 6 and 8 hours and otherwise stored at 8^oC kept for 12, 11 and 9 days respectively. A shelf life difference of approximately 3 days resulted from exposure differences of 4 hour periods.

Sodium propionate-treated samples exposed to room temperature for 4, 6 and 8 hour periods and otherwise stored at 8^oC kept for 16, 13 and 13 days, while those otherwise stored at 2^oC for 33, 29 and 26 days respectively (Table 2).

The differences in shelf life resulting from different exposure periods varied with treatments,but in almost all cases increased length of exposure period resulted in reduced storage life of cottage cheese.

In Trials 3 and 4, potassium sorbate-treated samples exposed to room temperature for 4, 6 and 8 hours kept for 30 days at $8^{o}C$, for 50 days at 2^{o} and $5^{o}C$. It was apparent that the shelf life of the potassium sorbate-treated samples was not affected by these periods of exposure to room temperature (Figures 6 and 7).

In Trial 5 (Figure 5), a comparison was made between the effects of an 8-hour exposure period on different days after finishing the cheese. The results (Table 3) showed no significant differences in storage life as a result of exposure to room temperature on the second, fourth or sixth day.

4. Microbial Tests.

The results revealed that microbial numbers varied widely within each group of microorganisms initially and after 14 days during storage at $5^{o}C$. For individual cottage cheese samples, there was no consistent relationship between the numbers of different groups of organisms. For example, a high standard plate count was not necessarily indicative of high numbers of coliform organisms or yeasts and molds.

In all cases standard plate counts, yeast and mold counts and psychrophile counts increased during storage. Coliforms were not observed in all samples, but if they were found initially they increased. For example, Trial 1 (Figure 8) showed that microbial counts of control samples rapidly increased while standard plate counts, yeast and mold counts and psychrophile

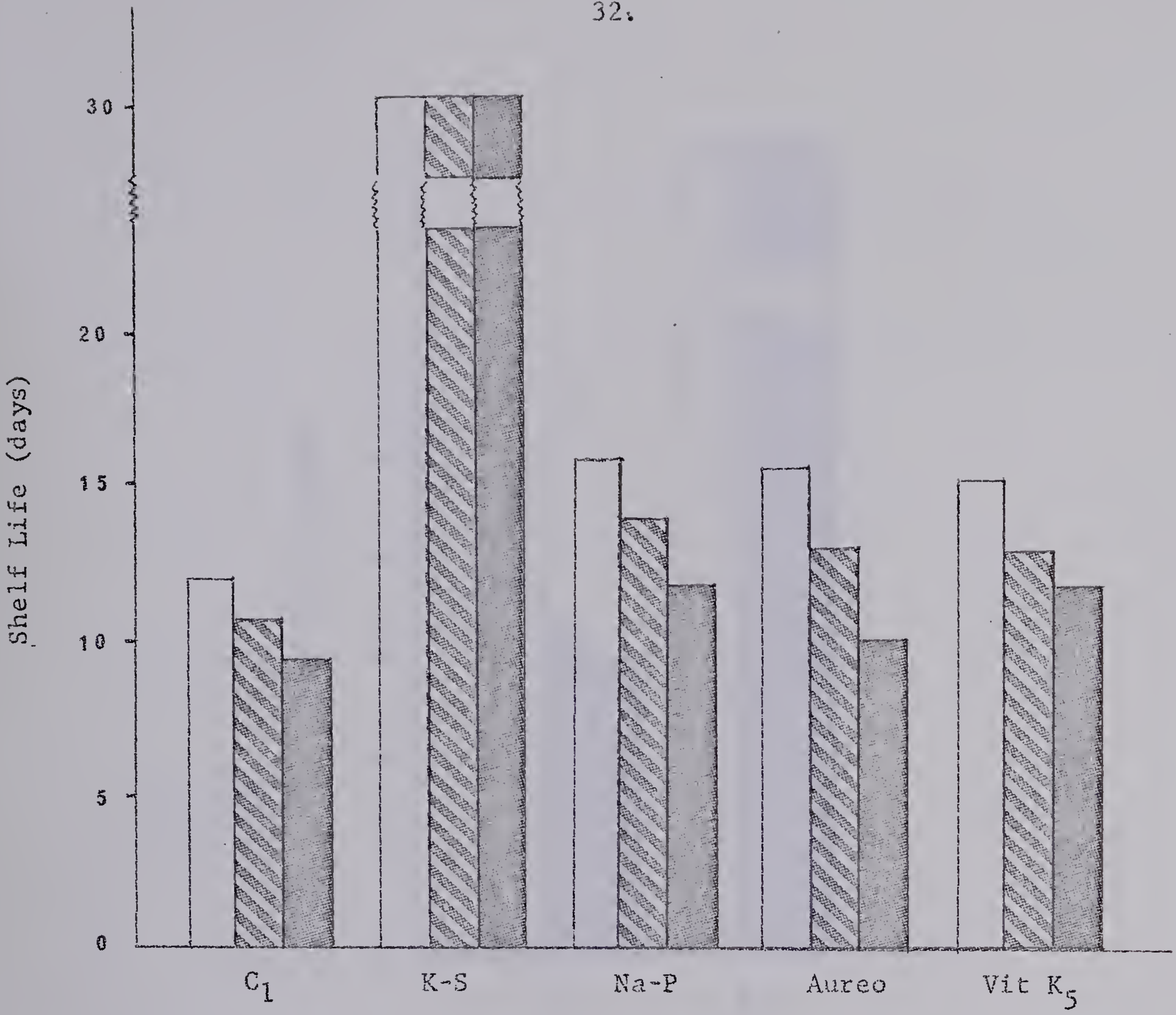


Figure 6. Shelf life of cottage cheese exposed to room temperature for different periods of time on the second day after manufacture and otherwise stored at 8°C (Trial 3).

C_1	:	Control (exposed)
K-S	:	Potassium sorbate 0.075%
Na-P	:	Sodium propionate 0.075%
Aureo	:	Aureomycin 6 ppm
Vit K_5	:	Vitamin K_5 15 ppm
□ (open)	:	4 hours
▨ (hatched)	:	6 hours
▩ (shaded)	:	8 hours

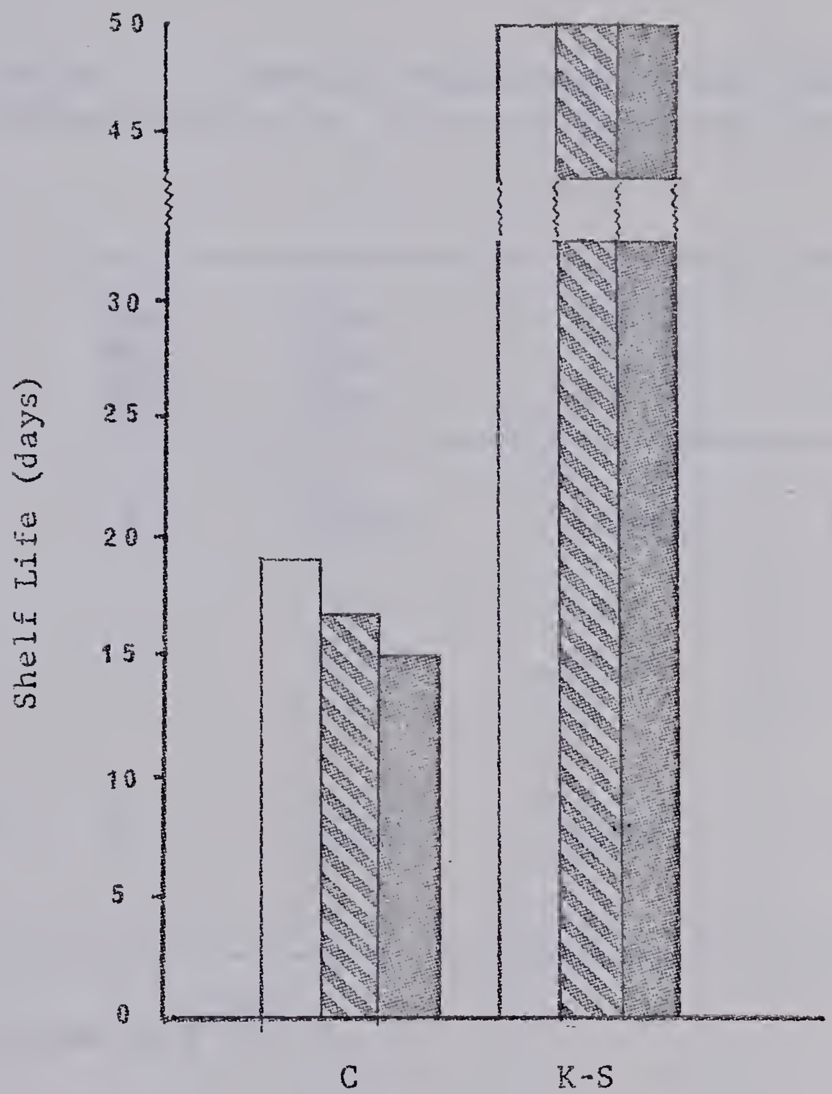


Figure 7. Shelf life of cottage cheese exposed to room temperature for different periods of time on the second day after manufacture and otherwise stored at 2 and 5 C (Trial 4)

C : Control

K-S : Potassium sorbate 0.050%.

: 4 hours

: 6 hours

: 8 hours

Table 3. Values of t showing comparisons of heat shock effect provided on different days (Trial 5).

Treatments	Heat Shock (hr.)	2nd. vs. 4th	2nd. vs. 6th	4th. vs. 6th
Control	8	0.967	1.360	1.414
Sodium-propionate (0.050%)	8	0.292	1.750	1.459
Potassium-sorbate (0.050%)	8	2.221	2.818*	1.600

* : Significant at $P^{0.05}$

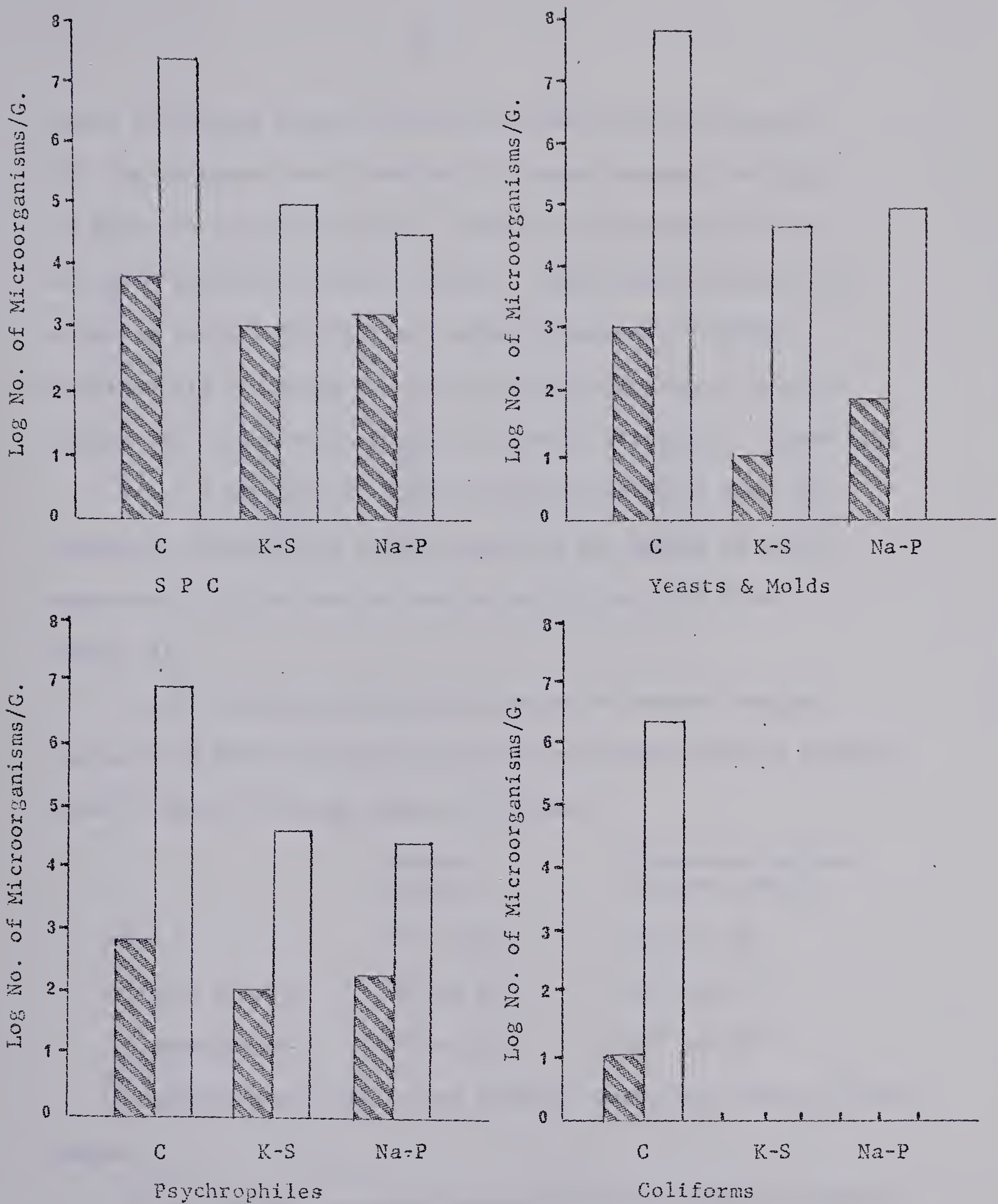


Figure 8. The microbial counts of cottage cheese initially and after 14 days storage at 5°C (Trial 1).

C : Control

K-S : Potassium sorbate 0.075%

Na-P : Sodium propionate 0.080%

: Initial

: 14 days

counts of treated samples slowly increased during storage at $5^{o}C$. No coliforms were observed in treated samples initially and after 14 days of storage. However, the microbial counts were much higher in control samples. This clearly showed that potassium sorbate (0.075%) and sodium propionate (0.080%) significantly inhibited the growth of microorganisms. Similar results were obtained in Trials 3, 4 and 5 (Figures 9, 10 and 11).

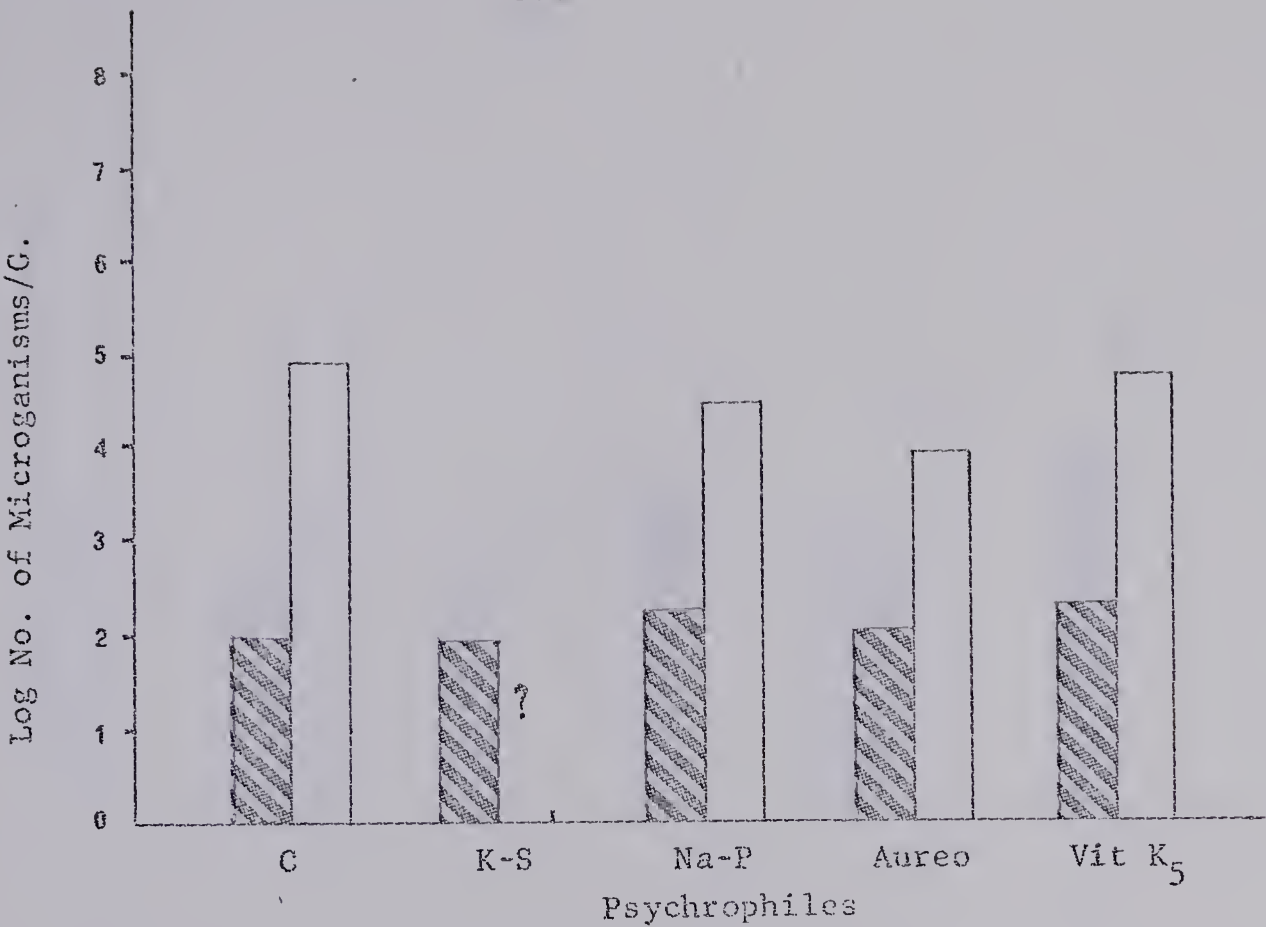
Trial 2 (Figure 12) showed that Aureomycin (6 ppm) and vitamin K_5 (15 ppm) had little effect on the growth of microorganisms. Similar results were shown in the third trial (Figure 9).

In all trials, the microbial counts of control samples reached much higher levels than potassium sorbate treated samples after 14 days of storage shown as follows:

	Control samples	Potassium sorbate treated samples
S P C	10^5 to 10^7	10^3 to 10^5
Yeasts & molds	10^4 to 10^7	10^2 to 10^5
Psychrophiles	10^4 to 10^7	10^2 to 10^5

Coliforms were usually not found in potassium sorbate treated samples.

It was evident potassium sorbate inhibited microbial growth more effectively than other inhibitor tested in this study. The results of shelf life tests showed potassium sorbate prolonged the shelf life of cottage cheese to a greater extent than any



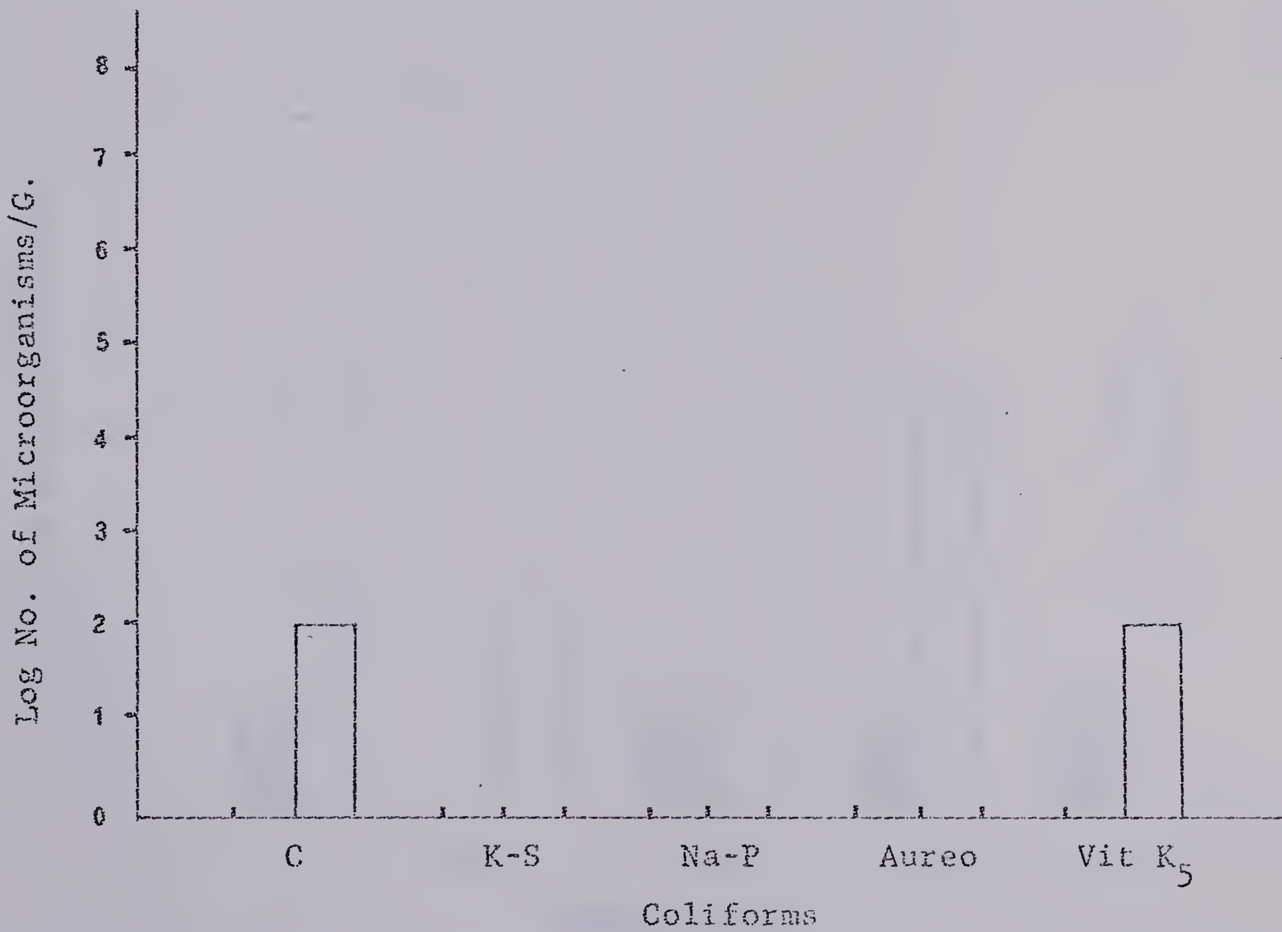


Figure 9. The microbial counts of cottage cheese initially and after 14 days storage at $5^{o}C$ (Trial 3).

C	:	Control
K-S	:	Potassium sorbate 0.075%
Na-P	:	Sodium propionate 0.075%
Aureo	:	Aureomycin 6 ppm
Vit K_5	:	Vitamin K_5 15 ppm

: Initial

: 14 days

? : Missing data

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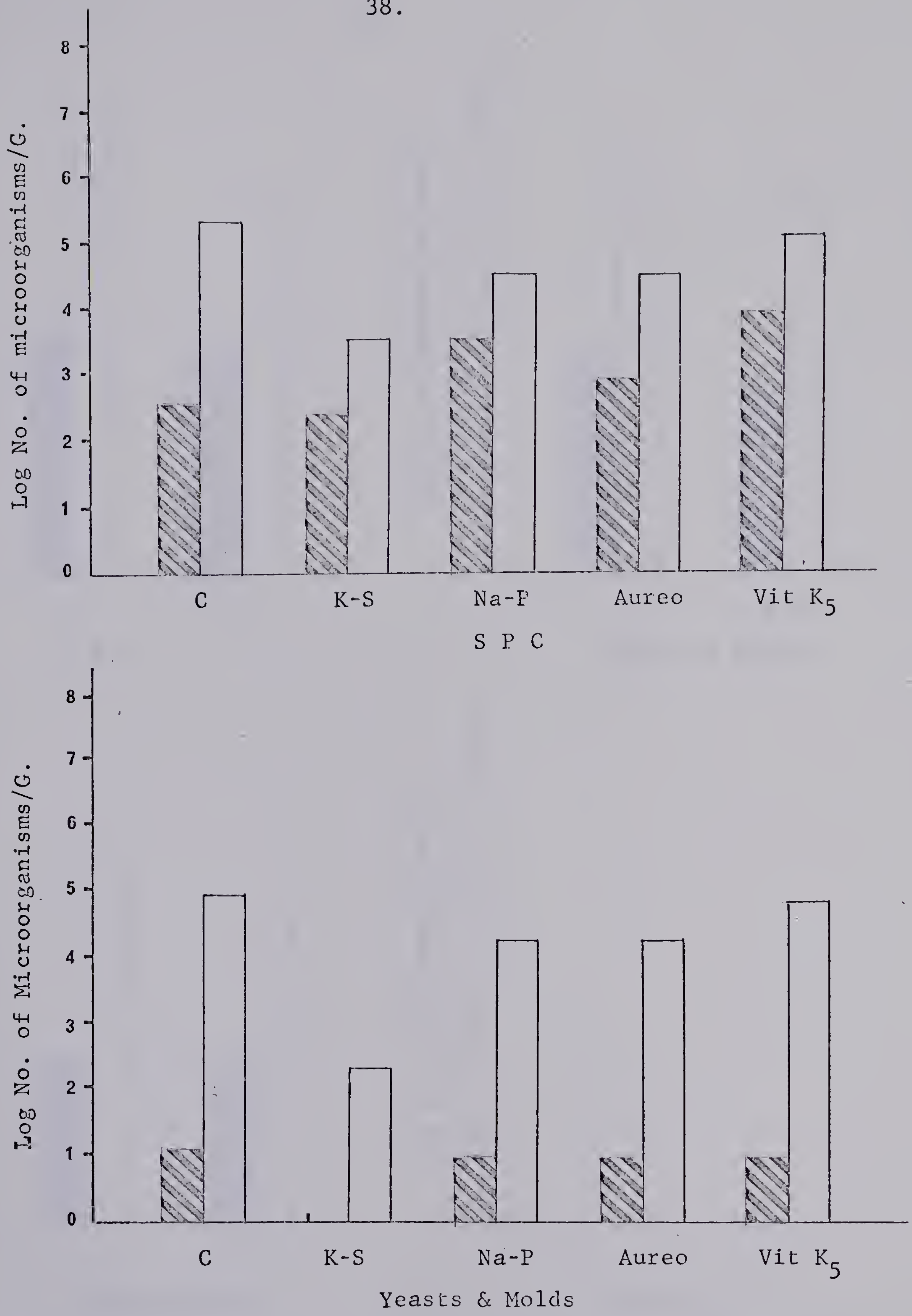

Figure 9. (continued) The microbial counts of cottage cheese initially and after 14 days storage at 5°C (Trial 3).

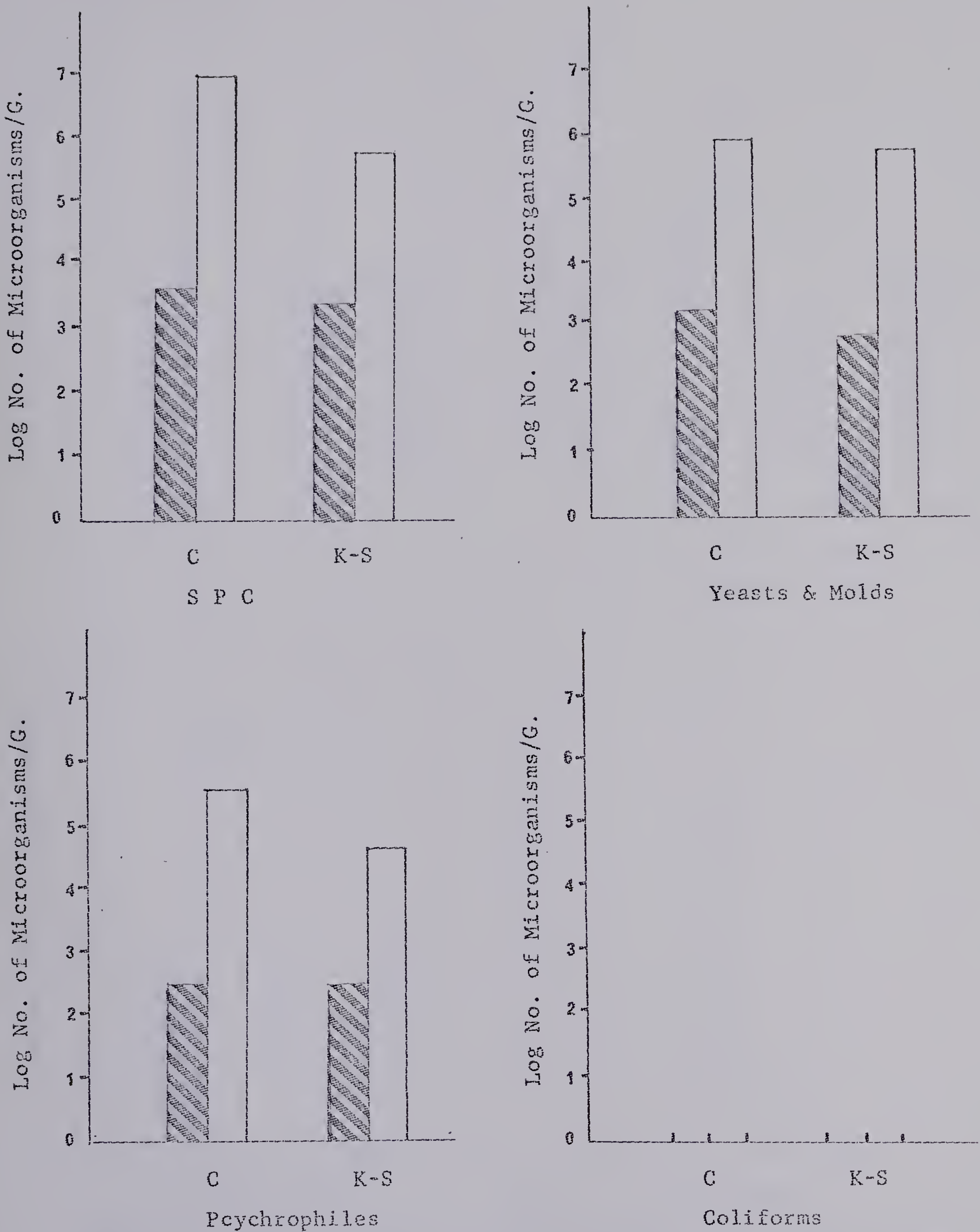

Figure 10. The microbial counts of cottage cheese initially and after 14 days storage at 5°C (Trial 4).

C : Control
K-S : Potassium sorbate 0.050%

: Initial
: 14 days

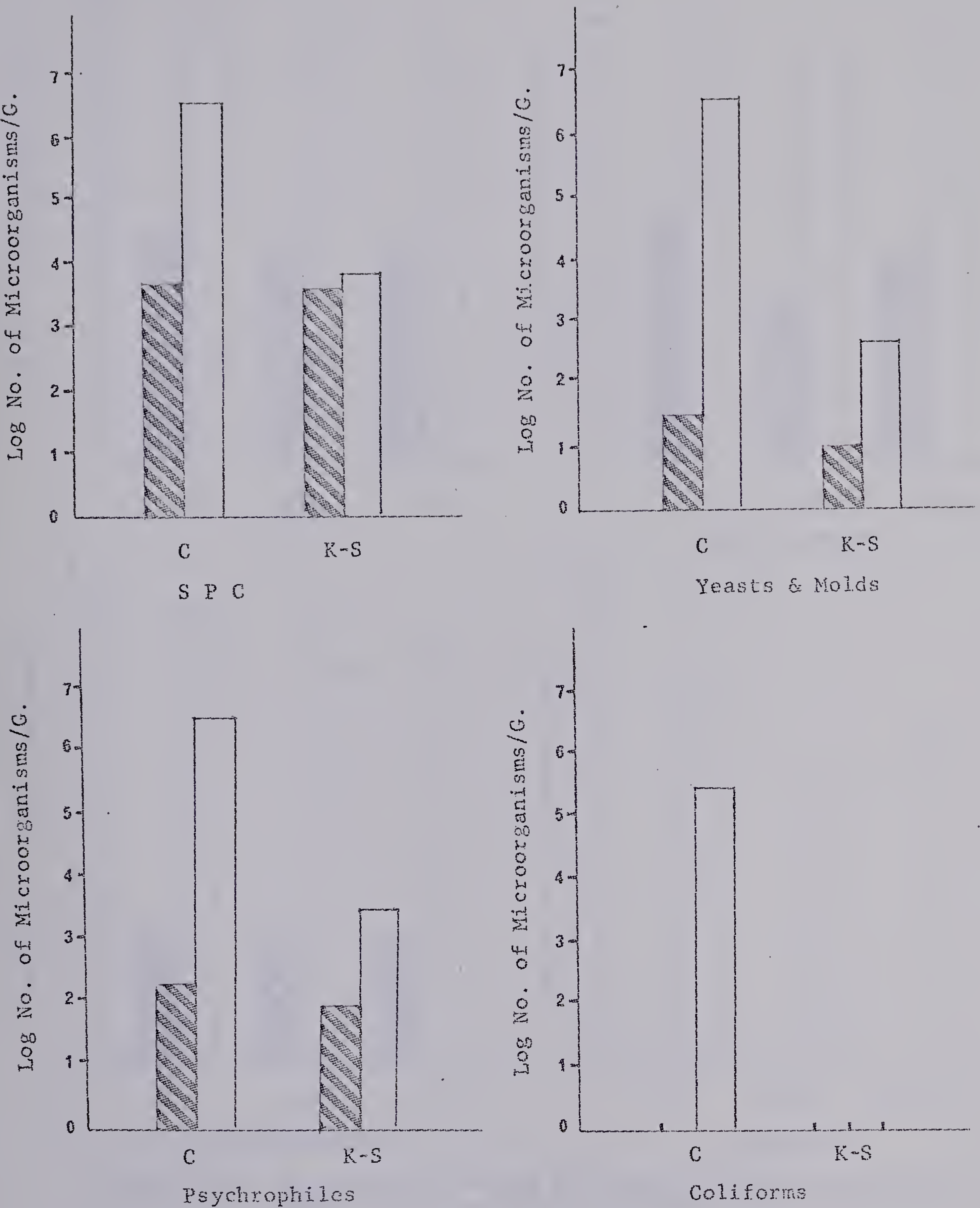

Figure 11. The microbial counts of cottage cheese initially and after 14 days storage at 5°C (Trial 5).

C : Control

K-S : Potassium sorbate 0.050%

: Initial

: 14 days

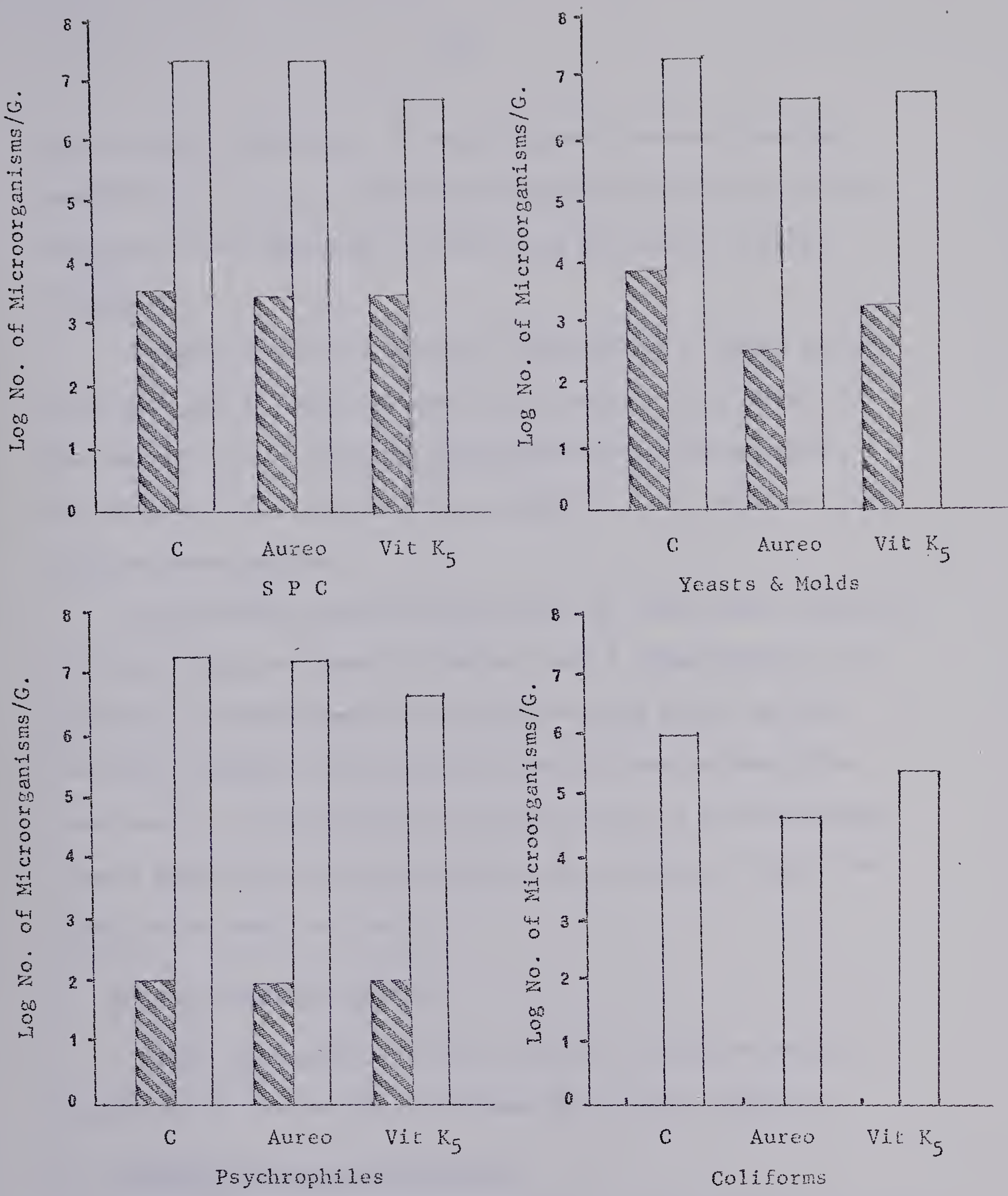

Figure 12. The microbial counts of cottage cheese initially and after 14 days storage at 5°C (Trial 2).

C : Control

Aureo : Aureomycin 6 ppm

Vit K_5 : Vitamin K_5 15 ppm

▨ : Initial

□ : 14 days

of the other inhibitors. It was therefore concluded that the shelf life of cottage cheese was prolonged by potassium sorbate because of its capability of inhibiting the growth of micro-organisms.

Because microbial tests were conducted on a single carton from each lot of cheese at each test period, a study (Trial 6) was carried out to learn the variation in count among cartons of the same lot of cottage cheese under the test conditions used in this investigation.

The microbial counts observed and the logarithmic transform of these counts are shown in Tables 4 and 5 respectively. For all lots, for each type of count and for each period of test the three randomly chosen cartons gave the same or nearly the same count. It was therefore considered that in this investigation a single carton can be accepted as an adequate sample from each lot for each test period.

5. pH and Titratable Acidity.

Little change was observed in pH and titratable acidity of any of the samples during storage for 14 days (Table 6).

6. Soluble Nitrogen Determination.

The development of bitter flavor in cottage cheese has been shown to be associated with proteolysis (43) and is as index of this change (44).

Table 4. Microbial counts of three different cartons from each treatment (Raw data) (Trial 6).

Microbial counts \ Cartons	Dry Curd			Cream			Potassium sorbate (0.050%)			Control creamed cottage cheese			Storage period (days)
	1	2	3	1	2	3	1	2	3	1	2	3	
Coliforms	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0		T N T C		0	0	0	0	0	0	7
	0	0	0				0	0	0	5×10^2	5×10^2	7×10^2	14
S P C	8×10^2	2×10^3	2×10^3	2×10^4	3×10^4	3×10^4	3×10^3	3×10^3	3×10^3	4×10^3	7×10^3	4×10^3	0
	1×10^4	2×10^4	1×10^4	4×10^6	9×10^6	5×10^6	4×10^3	4×10^3	4×10^3	2×10^5	2×10^5	1×10^5	7
	8×10^5	1×10^6	1×10^6	2×10^8	3×10^8	4×10^8	1×10^4	1×10^4	7×10^3	3×10^7	3×10^7	3×10^7	14
Yeasts & Molds	3×10	3×10	3×10	2×10^2	2×10^2	2×10^2	3×10	3×10	3×10	3×10	3×10	3×10	0
	2×10^4	2×10^4	4×10^4	3×10^6	4×10^6	4×10^6	2×10^3	2×10^3	2×10^3	2×10^5	1×10^5	9×10^4	7
	3×10^5	3×10^5	4×10^5	2×10^8	2×10^8	3×10^8	6×10^3	2×10^3	4×10^3	3×10^7	3×10^7	4×10^7	14
Psychrophiles	3×10^2	3×10^2	3×10^2	3×10^2	3×10^2	3×10^2	3×10^2	3×10^2	3×10^2	3×10^2	3×10^2	3×10^2	0
	1×10^4	2×10^4	1×10^4	1×10^5	7×10^6	5×10^6	7×10^2	5×10^2	7×10^2	2×10^5	1×10^5	1×10^5	7
	8×10^5	9×10^5	1×10^6	2×10^8	3×10^8	3×10^8	7×10^3	6×10^3	4×10^3	3×10^6	5×10^6	5×10^6	14

Table 5. Microbial counts of three different cartons from each treatment (Trial 6). (Logarithmic transform data)

Microbial counts \ Cartons	Dry Curd			Cream			Potassium sorbate (0.050%)			Control creamed cottage cheese			Storage period (days)
	1	2	3	1	2	3	1	2	3	1	2	3	
Coliforms	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0		T N T C		0	0	0	0	0	0	7
	0	0	0				0	0	0	2.699	2.699	2.845	14
S P C	2.903	3.301	3.301	4.301	4.477	4.477	3.477	3.477	3.477	3.602	3.845	3.602	0
	4.000	4.301	4.000	6.602	6.954	6.699	3.602	3.602	3.602	5.301	5.301	5.000	7
	5.903	6.000	6.000	8.301	8.471	8.602	4.000	4.000	3.845	7.477	7.477	7.477	14
Yeasts & molds	1.477	1.477	1.477	2.301	2.301	2.301	1.477	1.477	1.477	1.477	1.477	1.477	0
	4.301	4.301	4.301	6.477	6.602	6.602	3.301	3.301	3.301	5.301	5.000	4.954	7
	5.477	5.477	5.602	8.301	8.301	8.477	3.778	3.301	3.601	7.477	7.477	7.301	14
Psychrophiles	2.477	2.477	2.477	2.477	2.477	2.477	2.477	2.477	2.477	2.477	2.477	2.477	0
	4.000	4.301	4.000	5.000	6.845	6.699	2.845	2.699	2.845	5.301	5.301	5.000	7
	5.903	5.954	6.000	8.301	8.477	8.477	3.845	3.778	3.602	6.477	6.690	6.699	14

Table 6. pH and titratable acidity of cottage cheese initially and after 14 days storage at 5°C (Trials 1, 2, 3 and 5).

Tests	Treat-ments	Control		Potassium Sorbate (%)				Sodium propionate (%)				Aureomycin (ppm)		Vitamin K_5 (ppm)	
				0.050		0.075		0.050		0.075		6		15	
	Storage period	0	14	0	14	0	14	0	14	0	14	0	14	0	14
pH	Trial No 1.	5.10	5.12			4.96	4.99			5.09	5.07				
	2	5.10	5.11									5.09	5.11	5.12	5.10
	3	5.19	5.15			5.15	5.15	5.13	5.11	5.21	5.22	5.23	5.21	5.20	5.18
	5	5.11	5.11	5.10	5.09										
Titrat-able Acidity (%)	1	0.72	0.73			0.71	0.73			0.73	0.72				
	2	0.72	0.75												
	3	0.82	0.75			0.83	0.74			0.81	0.77	0.80	0.78	0.81	0.77

Changes observed in the percentage of water soluble nitrogen in cottage cheese with and without added potassium sorbate are shown in Table 7. The percentage of water soluble nitrogen increased more rapidly and reached higher values in the control samples than in the samples containing potassium sorbate (0.05%). This demonstrated that potassium sorbate was capable of inhibiting the growth of proteolytic microorganisms.

Table 7. Changes in the soluble nitrogen content of cottage cheese during storage (Trials 7 and 8).

Trial No.	Treatments	Soluble Nitrogen as per cent of Total Nitrogen *			
		Storage period			
		Initial	7 days	14 days	21 days
7	Control	9.37	10.14	10.98	
	Potassium sorbate (0.050%)	9.35	9.34	9.68	
8	Control	9.41	10.75	11.52	11.50
	Potassium sorbate (0.050%)	9.40	9.53	9.50	9.71

* Total nitrogen as per cent of cottage cheese was 2.13%.

DISCUSSION AND CONCLUSIONS

This study was planned primarily to learn if inhibitors could overcome the reduction in storage life that might be expected if cottage cheese was accidentally exposed to ambient temperature for a period as long as eight hours. Additional information was obtained on the value of inhibitors and lower storage temperatures in extending the storage life of cottage cheese held continuously under refrigeration.

The results indicated that all of the preservatives tested were capable at least of extending the shelf life of the cottage cheese to compensate for the loss caused by a heat shock of eight hours which was shown in Trial 3 to be about two days when the cottage cheese was otherwise stored at 5°C. Aureomycin, and vitamin K_5 at levels of 6 and 9 ppm and 15 and 20 ppm respectively were least effective in extending storage life. The effectiveness of potassium sorbate and sodium propionate was greater and these inhibitors more than compensated for the loss of storage life due to a heat shock.

A second main point indicated in the results is that temperature has a marked effect on storage life of cottage cheese. When no preservative was added, the storage life of the samples exposed to heat shocks varied with the temperatures at which they were held through the balance of their storage life.

Products held at 2°C had a longer storage life than those held at 5°C and both of these had longer storage life than those held at 8°C. In each case, holding the product at lower temperatures added 5 to 8 days to the storage life. It would appear therefore, that processors might obtain great advantages in storage life by holding their product at temperatures no higher than 2°C (35.6°F).

In this study, the growth of microorganisms on cottage cheese usually appeared as vivid pink spots, pink slime, or yellowish and brownish colored films. These were probably due to *Rhodotorula* spp., *Torolupsis* spp. and *Pseudomonas* spp. respectively. Also commonly found on the spoilage surface of cottage cheese were blue-green colored films, gray films and white gelatinous films probably due to *Penicillium* spp., *Mucor* spp., and *Alcaligenes metalcaligenes* respectively (19,22,25,35).

Potassium sorbate was the most effective inhibitor tested. Treated samples of cottage cheese had two to three times the storage life of untreated samples,and an eight hour exposure to room temperature had very little effect on the storage life of the treated product. It reduced the rate of growth of the three classes of microorganisms investigated in this study (coliforms, yeasts and molds, and psychrophiles), and it inhibited the activity of proteolytic microorganisms.

When used at the level of 0.100% it contributed a flavor

to the cottage cheese, but it was effective at levels of 0.050% and 0.075% and at these levels it had no effect on cottage cheese flavor.

Although potassium sorbate has not been approved by the Department of National Health and Welfare for use in cottage cheese, it has been approved for other food uses (e.g., amounts up to 0.2% may be used in process cheese) and its safety in diets has been thoroughly investigated. Demaree _et al_ (14) and Deuel _et al_ (15) showed that α, β unsaturated fatty acids were readily metabolized and that they would not interfere with the digestion of foods or cause any histopathological changes to occur in the anatomy of rats fed rations containing as much as 8% sorbic acid.

Manufacturers of cottage cheese in Alberta would like to be able to use a preservative in this product. However, we understand that processors in Ontario are satisfied with the present cottage cheese storage life. The different attitudes towards preservatives may be a result of the relatively small production and large distribution area in Alberta compared with the larger production and more concentrated market area in Ontario.

Obviously, the use of a preservative should in no way lessen the importance of using high quality skim milk, nor should it replace sanitary practices in manufacturing.

A longer shelf life should simply mean that higher quality cottage cheese could be made available to more people at reasonable prices. It might do much to increase consumption of this nutritious food.

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Appendix A. Values of t showing comparisons of heat shock effect (Trial 3).

Storage Temp (^{o}C)	Treat-ment	4 hr. vs. 6 hr.	4 hr. vs. 8 hr.	6 hr. vs. 8 hr.	0 hr. vs. 8 hr.
2^{o}	C_1	0.866	0.566	0.239	
	N_1	6.008**	12.969**	2.887*	
	N_2	7.857**	21.446**	6.114**	
	A_1	11.975**	20.778**	2.846*	
	A_2	8.083**	22.808**	7.273*	
	V_1	7.203**	5.669**	2.085	
	V_2	2.683*	7.155**	5.774**	
5^{o}	C_1				6.922** C_1 vs. C_2
8^{o}	C_1	3.794**	8.660**	5.692**	
	N_1	8.083**	11.547**	3.464**	
	N_2	5.367**	4.919**	0.577	
	A_1	5.367**	12.075**	8.660**	
	A_2	2.503*	8.854**	7.071**	
	V_1	4.264**	5.970**	2.309*	
	V_2	1.789	5.692**	2.357*	

** : Significant at $P^{0.01}$

* : Significant at $P^{0.05}$

C_1 : Control (exposed)

N_1 : Sodium propionate 0.075%

N_2 : Sodium propionate 0.100%

A_1 : Aureomycin 6 ppm

A_2 : Aureomycin 9 ppm

V_1 : Vitamin K_5 15 ppm

V_2 : Vitamin K_5 20 ppm

Appendix B. Periods of time in days to show evidence of microbial growth (Trials 1 and 2)

Storage Temp (^{o}C)	Trials No.		Control	K-sorbate 0.075%	Na-propopionate 0.080%	Aureomycin 6 ppm	Vitamin K_5 15 ppm
2^{o}	1	range	< 21	45-48	32-35		
		mean	< 21	47.2	33.6		
		S		1.30	1.51		
	2	range	17-18			20-21	20-22
		mean	17.8			20.8	21.2
		S	0.89			0.44	1.00
5^{o}	1	range	18-20	25-27	22-25		
		mean	19.4	26.6	23.2		
		S	0.89	0.94	1.30		
	2	range	15-17			16-18	16-19
		mean	15.8			17.2	17.4
		S	0.83			1.09	1.34
8^{o}	2	range	9-10			10-12	10-12
		mean	9.4			10.6	11.6
		S	0.54			0.89	0.89

S : Standard deviation from the mean.

Appendix C. Periods of time in days to show evidence of microbial growth (Trial 4).

Storage Temp (^oC)	Treatments		4 hr.	6 hr.	8 hr.	
		range	27-30	24-28	24-26	
	Control	mean	28.4	26.0	25.0	
		S	1.34	1.58	0.71	
2^o	Potassium	range	>50	>50	>50	
	sorbate (0.050%)	mean	>50	>50	>50	
		S				
		range	18-20	15-18	14-16	15-17
	Control	mean	19.2	17.0	15.4	16.6
		S	1.00	1.42	0.89	0.89
5^o	Potassium	range	> 50	>50	>50	
	sorbate (0.050%)	mean	>50	>50	>50	
		S				
		range	13-14	10-11	9-10	
8^o	Control	mean	13.2	10.4	9.4	
		S	0.45	0.55	0.55	

S : Standard deviation from the mean.

Appendix D. Periods of time in days to show evidence of microbial growth (Trial 5).

Treatments		2nd day	4th day	6th day
Control	range	13-16	12-18	13-18
	mean	15.0	16.2	16.4
	S	1.23	2.49	1.95
Sodium propionate (0.050%)	range	16-21	17-22	18-24
	mean	18.8	19.2	21.2
	S	2.17	2.17	2.17
Potassium sorbate (0.050%)	range	50-56	48-51	44-48
	mean	52.6	48.8	47.2
	S	2.60	1.30	1.79

S : Standard deviation from the mean

Appendix E. Microbial counts of cottage cheese during storage at 5°C.

Treatments	Storage period (days)	S P C	Psychro-philes	Yeasts & Molds	Coliforms
Trial 1.					
Control		6×10^3	6×10^2	1×10^3	1×10
K-sorbate	0	1×10^3	$<1 \times 10^2$	$<1 \times 10$	0
Na-propionate		2×10^3	$<2 \times 10^2$	$<1 \times 10^2$	0
Control		2×10^5	2×10^5	4×10^4	1×10^3
K-sorbate	7	4×10^3	4×10^3	8×10^2	0
Na-propionate		7×10^3	1×10^4	2×10^2	0
Control		2×10^7	1×10^7	6×10^7	2×10^6
K-sorbate	14	8×10^4	5×10^4	5×10^4	0
Na-propionate		4×10^4	3×10^4	8×10^4	0
Trial 2.					
Control		4×10^3	$<1 \times 10^2$	6×10^3	0
Aureomycin	0	3×10^3	$<1 \times 10^2$	3×10^2	0
Vitamin K_5		3×10^3	$<1 \times 10^2$	2×10^3	0
Control		4×10^5	3×10^5	3×10^5	6×10^5
Aureomycin	7	5×10^5	3×10^5	3×10^5	$<3 \times 10^2$
Vitamin K_5		3×10^5	2×10^5	2×10^5	2×10^3
Control		2×10^7	2×10^7	2×10^7	1×10^6
Aureomycin	14	2×10^7	2×10^7	5×10^6	5×10^4
Vitamin K_5		6×10^6	5×10^6	6×10^6	3×10^5
Trial 4.					
Control	0	4×10^3	$<3 \times 10^2$	2×10^3	0
K-sorbate		3×10^3	$<3 \times 10^2$	8×10^2	0
Control	14	$>1 \times 10^7$	4×10^5	$>1 \times 10^6$	0
K-sorbate		9×10^5	7×10^4	$>8 \times 10^5$	0

Appendix E. (continued) Microbial counts of cottage cheese during storage at 5°C.

Treatments	Storage period (days)	S P C	Psychro-philes	Yeasts & Molds	Coliforms
<u>Trial 3</u>.					
Control		3×10^2	$<1 \times 10^2$	$<1 \times 10$	0
Aureomycin 1		9×10^2	$<1 \times 10^2$	$<1 \times 10$	0
Aureomycin 2		2×10^3	$<1 \times 10^2$	$<1 \times 10$	0
K-sorbate 1		2×10^2	$<1 \times 10^2$	0	0
K-sorbate 2	0	3×10^2	$<1 \times 10^2$	0	0
Na-propionate 1		3×10^3	$<2 \times 10^2$	$<1 \times 10$	0
Na-propionate 2		3×10^3	$<2 \times 10^2$	$<1 \times 10$	0
Vitamin K_5 1		4×10^2	$<2 \times 10^2$	$<1 \times 10$	0
Vitamin K_5 2		8×10^3	$<2 \times 10^2$	$<1 \times 10$	0
Control		2×10^5	9×10^4	9×10^4	$<1 \times 10^2$
Aureomycin 1		3×10^4	4×10^4	2×10^4	0
Aureomycin 2		5×10^4	2×10^4	2×10^4	0
K-sorbate 1		3×10^3	—	2×10	0
K-sorbate 2	14	3×10^3	—	2×10	0
Na-propionate 1		3×10^4	3×10^4	2×10^5	0
Na-propionate 2		5×10^4	6×10^4	5×10^4	0
Vitamin K_5 1		5×10^4	3×10^4	4×10^4	$< 5 \times 10$
Vitamin K_5 2		1×10^5	6×10^4	9×10^4	$< 1 \times 10^2$
<u>Trial 5</u>.					
Control		5×10^3	2×10^2	$<3 \times 10$	0
K-sorbate	0	4×10^3	1×10^2	$<1 \times 10$	0
Na-propionate		4×10^3	1×10^2	$<2 \times 10$	0
Control		6×10^3	4×10^3	4×10^3	3×10^2
K-sorbate	7	5×10^3	7×10^2	3×10^2	0
Na-propionate		5×10^3	9×10^2	6×10^2	0
Control		4×10^6	4×10^6	4×10^6	3×10^5
K-sorbate	14	7×10^3	3×10^3	5×10^2	0
Na-propionate		5×10^4	6×10^4	1×10^5	0